REMARKS

Applicant thanks Examiner Afremova for the courtesy of a personal interview on June 14, 2006, with the undersigned attorney, Steven J. Frank, and Marlo T. Walpole. During the interview, proposed claim amendments and the prior art were discussed.

Claims 1, 2, 4, 6-16, 20-22, 30, 31, 36-40, 42-44, 48-53, 116-120, 122, and 123 have been amended without any intention of disclaiming equivalents thereof. Support for the amendments is found in the specification, for example, on page 18, line 28 to page 19, line 8 and on page 27, line 16 to page 28, line 2. It is respectfully submitted that no new matter is added. Accordingly, after entry of this amendment, claims 1-4, 6-23, 30-40, and 42-123 are pending, and claims 57-115 are withdrawn from consideration.

Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 1, 38, and 116 presently stand rejected under 35 U.S.C. §112, second paragraph for being indefinite with regard to the term "predegenerating." It is respectfully submitted that the meaning of this term is clear in view of the specification and meaning in the art. Specifically, on page 6, lines 11-15 of the specification, *in vivo* predegeneration and its role in supporting nerve regeneration is described. As mentioned in the specification, it is impractical to create predegenerated nerves in humans because it would involve nerve injury followed by a period of survival *in vivo* to allow tissue degeneration. In degeneration, cellular and molecular mechanisms act to enhance the growth-promoting properties of the basal lamina which then retains the ability to stimulate nerve regeneration after cellular elements have been killed. (Specification, page 7, lines 21-24.) *In vitro* predegeneration, however, is practical and results in a substantial increase in the growth-promoting ability of acellular nerve grafts. (Specification, page 7, lines 24-26.) For example, culture methods of the invention involve predegenerating nerve tissue *in vitro*, which, following engraftment, improves the ability of regenerating neurons to traverse the interface between the graft and the host nerve tissue. (Specification, page 27, lines 17-20.) Without being bound by any particular theory or mechanism, it is believed that the culturing methods of the subject invention allow the living nerve cells to express chondroitin sulfate proteoglycan-degrading enzymes (CSPG-degrading enzymes) and promote Schwann cell proliferation, as would occur naturally *in vivo* during the remodeling process of nerve degeneration. (Specification, page 27, lines 20-23.) As mentioned on page 28, lines 18-20, the

methods can also include application of exogenous CSPG-degrading enzymes. In fact, in Example 17 of the application, *in vivo* and *in vitro* predegeneration are experimentally compared. Accordingly, predegenerating has a clear meaning in view of the specification and the art such that predegenerating conditions (i.e., those conditions resulting in predegeneration) also has a clear meaning, and Applicant respectfully traverses this rejection.

Claims 1, 38, and 116 presently stand rejected under 35 U.S.C. §112, second paragraph for being indefinite with regard to the term "nerve tissue." As discussed with Examiner Afremova, Applicant has amended the claims to recite a nerve graft, rather than nerve tissue, where the nerve graft comprises a nerve tissue segment. Accordingly, Applicant requests reconsideration and withdrawal of this rejection.

Claims 1, 6, 38, and 116 presently stand rejected under 35 U.S.C. §112, second paragraph for being indefinite with respect to the recitation of "subsequently implanted." Claims 1, 6, 38, and 116, as amended, clarify that the increase in neurite-promoting activity of the nerve graft and/or the increase in axon ingress and extent of growth within the nerve graft occurs when the nerve graft is in use. Accordingly, Applicant requests reconsideration and withdrawal of this rejection.

Claims 2-4 presently stand rejected under 35 U.S.C. §112, second paragraph for being indefinite and incomplete. Claims 2-4, as amended, clarify that the method further comprises performing an *in vitro* or *in vivo* neurite outgrowth assay of the nerve graft to determine the increase in neurite-promoting activity. Accordingly, Applicant requests reconsideration and withdrawal of this rejection.

Rejections Under 35 U.S.C. §102

The La Fleur Reference

Claims 1-4, 6-15, 17-21, 30-51, 53-56, and 116-123 stand rejected over La Fleur *et al.* (1996), J. Exp. Med. 184: 2311-2326 ("La Fleur") under 35 U.S.C. §102(b). La Fleur describes a study intended to identify major MMPs and TIMPs involved in repair after peripheral nerve injury and the possibility that protection of basement membrane (BM) from proteolytic degradation is a relevant mechanism during repair of injury to nerve. (Page 2312, left column.) La Fleur found that, in response to a crush injury, TIMP-1 was induced. (Abstract.) TIMP-1

protected BM type IV collagen from degradation by exogenous MMP-9 in cryostat sections of nerve *in vitro*. (*Id.*) La Fleur concludes that TIMP-1 may protect BM from uncontrolled degradation after injury. (*Id.*) More specifically, La Fleur suggests that, *in vivo*, TIMP-1 protects specific BM components of injured nerve from degradation by MMPs. (Page 2322, left column.)

La Fleur teaches essentially the opposite of the present invention. Whereas Applicant's understanding of predegeneration involves degradation of CSPG, La Fleur teaches that TIMP-1 preserves CSPGs by protecting against MMPs during *in vivo* degeneration (*i.e.*, Wallerian degeneration). The fact that La Fleur teaches that TIMP-1 protects basement membrane from degradation by MMPs (page 2322, left column), particularly during Wallerian degeneration to promote axonal regrowth *in vivo* (page 2323, right column), indicates that La Fleur's mechanism would not increase neurite-promoting activity of the nerve graft as required by the present claims. Thus, not only does La Fleur fail to teach predegenerating conditions or conditions permissive to degeneration and remodeling of the nerve graft, but in fact — in preserving CSPGs through TIMP-1 rather than degrading CSPGs — teaches behavior diametrically opposed to that claimed herein. Certainly La Fleur does not describe predegenerating conditions or conditions permissive to degeneration and remodeling of the nerve graft.

For these reasons, amended independent claims 1, 38, and 116 are novel over La Fleur. Claims 2-4, 6-15, 17-21, 30-37, 39-51, 53-56, and 117-123, which depend directly or indirectly from an allowable base claim, also are allowable. Applicant respectfully requests that this rejection be reconsidered and withdrawn.

The Dennis Reference

Claims 1-4, 6-15, 17-21, 30-32, 34-40, 42-45, 47-51, 53-56, 116, 119, 122, and 123 stand rejected over U.S. Patent No. 6,448,076 to Dennis *et al.* ("Dennis") under 35 U.S.C. §102(e). Dennis reports on a method of acellularization. Briefly, rat peripheral nerve segments are surgically removed, pinned at slack length within a culture dish, and immediately submersed in Dulbecco's Phosphate Buffered Saline (PBS). Then, the acellularization method is carried out at room temperature. (Col. 3, lines 34-50.) These acellularized nerve grafts reportedly support axonal regeneration and allow end-organ reinnervation. (Col. 6, lines 21-24.)

For the reasons that follow, Applicant respectfully submits that the rejected claims are neither anticipated nor even suggested by Dennis. In particular, amended independent claim 1 recites, in part, culturing a nerve graft *in vitro* under predegenerating conditions that remodel the nerve graft and that increase neurite-promoting activity when the nerve graft is in use; amended independent claim 38 recites, in part, culturing a nerve graft *in vitro* under predegenerating conditions that increase neurite-promoting activity when the nerve graft is in use; and amended independent claim 116 recites, in part, placing a nerve graft in conditions *in vitro* that are permissive to the degeneration and remodeling of the nerve graft *in vitro* and that increase neurite-promoting activity when the nerve graft is in use.

With regard to amended independent claims 1, 38, and 116, Dennis does not describe a culturing step or a placing step under the permissive conditions recited in claim 116. In Dennis, the nerve is placed in PBS and then acellularization is carried out. There is no culturing step or a placing step that would necessarily fulfill the express requirements of claim 116 with regard to cell activation and proliferation, enzyme activity, and degeneration and remodeling.

Furthermore, with regard to amended independent claims 1 and 38, Dennis does not describe predegenerating conditions that increase the neurite-promoting activity of the nerve graft, and, with regard to amended independent claim 116, Dennis does not describe conditions that are permissive to degeneration of the nerve graft and increase the neurite-promoting activity of the nerve graft. As mentioned above, predegenerating conditions under certain circumstances activate CSPG-degrading enzymes (specification, page 27, lines 20-23) and/or involve addition of CSPG-degrading enzymes (specification, page 28, lines 18-20 and Example 3). These conditions increase the neurite-promoting activity of the nerve graft. Although Dennis does describe nerve grafts that support axonal regeneration, it does not follow that predegenerating or degenerating conditions are present. This is demonstrated, for example, by Applicant in Examples 3, 12, and 18. In Example 3, the treated nerve grafts had a decreased latency and improved accession of axonal regeneration into acellular nerve grafts. However, Figure 5 shows that some axonal ingrowth was seen in all (treated and control) grafts, and Figure 6 shows that all samples (treated and control) showed axonal penetration. While the treated graft showed a better response, the control grafts showed at least some response. Example 12 shows something similar to Example 3. As described in Example 12 and Figures 15A and B, neurite growth of

DRG neurons on nerve explants cultured for 2 days in 2% serum (an example of *in vitro* predegeneration) was 70% greater than control nerves (not predegenerated.) However, there was some outgrowth even in the control nerves. Finally, Example 18 and Figures 20A and B also indicate that axonal regeneration into acellular nerve grafts is enhanced by *in vitro* predegeneration, but that axonal growth occurred within the basal lamina tubes in both the predegenerated and control conditions.

Thus, it is improper to infer predegeneration from a graft that supports axonal regeneration. In effect, the Examiner seems to view predegenerating conditions as inherent, given the result of axonal regeneration. This is incorrect not only as a factual matter, as described above, but legally as well: the Federal Circuit has repeatedly held that the relevance of a reference cannot be predicated on "mere conjecture." In re Robinson W.L. Gore & Associates, Inc. v. Garlock, Inc., 721 F.2d 1540, 220 U.S.P.Q. 303 (Fed. Cir. 1983), cert. denied, 469 U.S. 851, 105 S.Ct. 172 (1984); Continental Can Co. v. Monsanto Co., 948 F.2d 1264, 1268, 20 U.S.P.Q.2d 1746, 1749 (Fed. Cir. 1991) ("Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient").

Moreover, amended independent claims 1, 38, and 116 all require enhancement of neurite-promoting activity resulting from or in combination with predegeneration/degeneration. Dennis does not show an *increase* in the neurite-promoting activity of a nerve graft, just that its nerve grafts support axonal regeneration.

Accordingly, for the reasons provided above, amended independent claims 1, 38, and 116 are novel over Dennis. Claims 2-4, 6-15, 17-21, 30-32, 34-37, 39, 40, 42-45, 47-51, 53-56, 119, 122, and 123, which depend directly or indirectly from an allowable base claim, also are allowable. Applicant respectfully requests that this rejection be reconsidered and withdrawn.

The Lassner Reference

Claims 1-4, 6-15, 17-23, 30-40, 42-56, and 116-123 stand rejected over Lassner *et al.* (1995), *J. Reconstructive Microsurgery* 11(6):447-453 ("Lassner") under 35 U.S.C. §102(b). Lassner investigated methods of preserving peripheral nerve grafts. The paragraph bridging columns 1 and 2 of page 448 of Lassner describes three experimental groups: nerve segments

placed in cold storage at 4 °C under ischemic conditions for periods of 14 hours, 32 hours, 72 hours, or 120 hours (Groups A-H); normal animal controls having the nerve dissected, left in the animal, and subsequently sutured in the absence of extracorporeal pretreatment (Group K), and negative controls where nerves were subjected to repeated freezing and thawing to evacuate all viable cells (Group I). Therefore, the nerve sections were (1) removed from the animal and stored in cold, ischemic conditions prior to implantation, (2) left in the animal after nerve dissection and subsequently sutured, or (3) removed from the animal and rendered acellular prior to implantation.

As discussed with the Examiner during the interview, these activities do not rise to the level of culturing, much less predegenerating. The cold, ischemic conditions are stasis conditions and do not promote physiological activity. In Applicant's system, if there is no physiological activity, there is no remodeling of a nerve segment (e.g., by degrading CSPGs) and, thus, no predegenerating conditions. Predegenerating conditions lead to an increase the neurite promoting activity of a nerve graft when it is in use. Thus, Lassner's stasis conditions are not culturing conditions, and certainly they are not predegenerating conditions or conditions permissive to degeneration and remodeling. Evacuating the viable cells in the nerve can be done without culturing or placing the nerve graft under the permissive conditions recited in claim 116, as reflected, for example, in the amended independent claims.

Furthermore, leaving the severed nerve in the animal is not an *in vitro* treatment and, thus, is not relevant to the amended independent claims that involve *in vitro* nerve graft treatment. In none of the three described dispositions of nerve segments does Lassner disclose or even suggest culturing a nerve graft *in vitro* under predegenerating conditions (claims 1 and 38) and/or placing a nerve graft in conditions *in vitro* that are permissive to the degeneration and remodeling of the nerve graft *in vitro* (claim 116).

In a second series of experiments, nerve grafts were prepared as described above, dissected into small segments, placed in a culture dish containing Dulbecco's Modified Eagle Medium, and maintained at 5% CO_2/95% air for two days. The tissue segments were then evaluated morphologically, fixed with methanol at -18 °C, and immunohistologically stained without any reimplantation. Thus, these experiments are merely histological in nature and not a

method for preparing a nerve graft (claims 1 and 116) or a method for enhancing the regenerative potential of a nerve graft (claim 38).

Additionally, the described conditions do not necessarily result in an increase in the neurite-promoting activity of the nerve graft. As explained above in connection with Dennis, it is improper to infer predegeneration from any particular set of conditions. Predegenerating conditions or conditions permissive to degeneration and remodeling result in an increase in neurite-promoting activity of a nerve graft.

Accordingly, for the reasons provided above, amended independent claims 1, 38, and 116 are novel over Lassner. Claims 2-4, 6-15, 17-23, 30-37, 39, 40, 42-56, and 117-123, which depend directly or indirectly from an allowable base claim, also are allowable. Applicant respectfully requests that this rejection be reconsidered and withdrawn.

Rejection Under 35 U.S.C. §103(a)

Claims 1-4, 6-23, 30-40, 42-56, and 116-123 stand rejected over Dennis, La Fleur, Ide *et al.* (1983), Brain Research 288:61-75 ("Ide"), and Evans *et al.* (1994), Prog. Neurobiology, 43:187-233 ("Evans") under 35 U.S.C. §103(a). As mentioned above, both Dennis and La Fleur fail to describe methods for preparing a nerve graft or for enhancing the regenerative potential of a nerve graft involving culturing a nerve graft *in vitro* under predegenerating conditions that increase neurite-promoting activity when the nerve graft is in use or involving placing a nerve graft in conditions *in vitro* that are permissive to the degradation and remodeling of the nerve graft *in vitro* and that increase neurite-promoting activity when the nerve graft is in use. Neither Ide nor Evans cures the deficiency of Dennis or La Fleur as the former are directed to *in vivo* predegeneration. Evans additionally reports that predegeneration has no clinical relevance, teaching away from any combination. (Page 212.)

Accordingly, for the reasons provided above, amended independent claims 1, 38, and 116 are patentable over Dennis, La Fleur, Ide, and/or Evans. Claims 2-4, 6-23, 30-37, 39, 40, 42-56, and 117-123, which depend directly or indirectly from an allowable base claim, also are allowable. Applicant respectfully requests that this rejection be reconsidered and withdrawn.

CONCLUSION

In view of the foregoing, Applicant respectfully requests that the foregoing rejections be reconsidered and withdrawn. The Examiner is invited to contact the undersigned attorney with any questions about this submission. Early favorable action is respectfully solicited.

Respectfully submitted,

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